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Function of Topoisomerase II and the Consequences of Inhibition

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INTRODUCTION

Topoisomerases are cellular enzymes essential for normal DNA metabolism. Topoisomerases play a major role in two important aspects of pediatric oncology. First, they are essential for the action of some of the most effective cytotoxic drugs, the topoisomerase II inhibitors. Second, because of their ability to create strand breaks in DNA, they may be involved in the etiology of some leukemias. This review will discuss the biology of topoisomerases, the role they play in the action of some cytotoxic drugs, and alterations in topoisomerases that may be important in the development of drug resistant disease. Possible mechanisms to explain the role of topoisomerases in the causation of DNA damage leading to therapy-related secondary leukemia will also be discussed.

TOPOISOMERASES: BIOLOGY

Topoisomerases are cellular enzymes that can change the topological conformation (shape) of DNA by introducing double or single strand breaks into the double helix [1,2]. The need for such an activity arises from the fact that in eukaryotic cells chromosomes are not simply free-floating strands of DNA. Each human cell contains a length of 4 inches of DNA; and in order to package this material into a cell, the DNA exists as chromatin. Chromatin consists of DNA that is looped and bound to protein to form units known as nucleosomes. The binding of DNA to protein means that each loop of DNA effectively has fixed ends. This causes difficulties during processes such as transcription and replication that require separation of the two strands of the DNA double helix, unless the DNA is able to "swivel." Each loop of DNA can be imagined as a piece of rope made of up two strands twisted together with each end held tight in a vise. When the two strands are pulled apart, separating the two strands is impossible. Partial separation can only be achieved by tightening the twisting at each end of the rope (Fig. 1). If the strands of the rope are cut and then pulled apart, the strands can be separated without tightening the twisting at either end. Metabolic events such as transcription and replication can then take place, and the break can be rejoined (Fig. 1). In addition to allowing DNA to "swivel," topoisomerases are able to perform an "unknotting" function, allowing the untangling of intertwined loops of DNA [3]. The importance of this aspect of topoisomerase activity is illustrated by the demonstration that yeast cells that lack topoisomerase activity become fixed in anaphase during mitosis, unable to segregate their tangle of newly replicated chromosomes [4–6].

Topoisomerases are classified into two groups, type I and type II. Type I topoisomerases cause a break in one strand of the DNA double helix and type II topoisomerases cause a break in both strands [1,2]. Both types of topoisomerase achieve DNA single or double strand breakage and rejoining in a similar way (illustrated for topoisomerase II in Fig. 2). In the first step, the enzyme binds to DNA, forming a phosphotyrosyl bond with the 5′ ends of each strand, and introducing a break into the sugar and phosphate backbone of the DNA (Fig. 2A). Topoisomerase II is a homodimer with two identical subunits. Once a break has been made in the DNA backbone, the two topoisomerase subunits can separate leaving a gap, or DNA strand break (Fig. 2B). The gap in the DNA can act as a "swivel," allowing relaxation of torsional stress as described earlier, or another DNA helix can pass through the gap, allowing the enzyme to perform an "unknotting" function to resolve tangles in DNA. In the final step of the reaction, the two topoisomerase monomers come together and reseal the DNA backbone. The enzyme then separates from the DNA and is free to start another reaction (Fig. 2C).

Cleavage of DNA by topoisomerase II does not occur randomly throughout the genome; DNA binding and cleavage is most marked at preferred sites. Sequencing of the preferred sites of cleavage has identified a so-called consensus sequence for topoisomerase II binding and DNA breakage [7]. This sequence is 18 bases long

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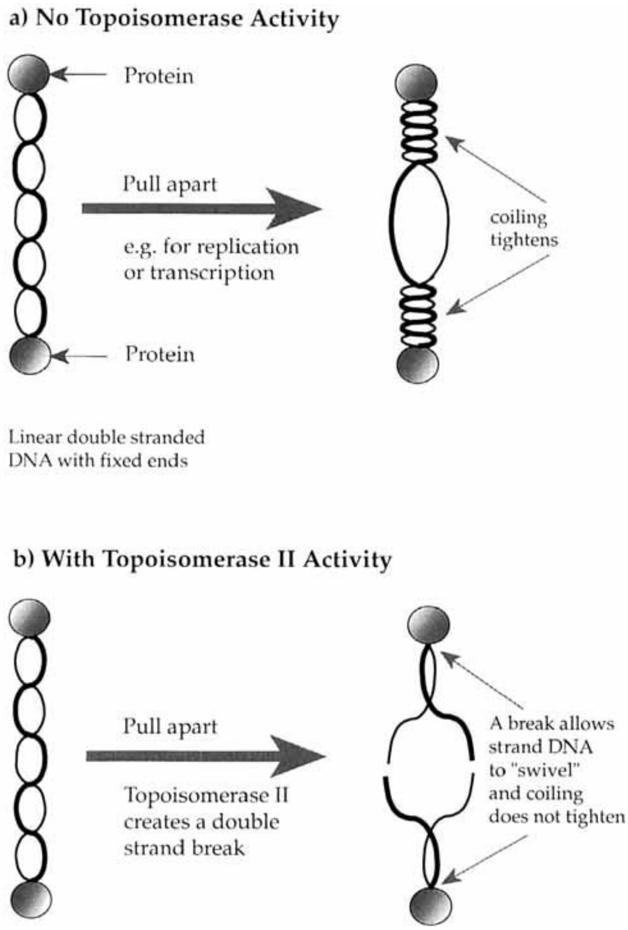

Fig. 1. a,b: Topoisomerase activity is essential for the relaxation of torsional stress during DNA metabolism.

and is degenerate, that is 8 of the 18 bases are N, where N is any base and 6 of the remaining 10 bases can be either of 2 bases. The vertebrate consensus sequence was derived in vitro using cleavage patterns generated in naked DNA, a model system very different from the in vivo situation where substrate DNA is protein bound and topologically constrained as nucleosomes. These different conditions may have a profound effect on the ability of topoisomerase to bind and cleave DNA and this should be remembered when using this sequence to identify evidence of topoisomerase II activity in vivo. Indeed, there is in vivo evidence that chromatin conformation is more significant than DNA sequence in determining topoisomerase cleavage sites, with cleavage preferred at easily accessible internucleosomal sites [8]. The need for caution in the use of consensus sequences is emphasized by studies of the Drosophila topoisomerase II enzyme, where investigators have shown that the cleavage consensus sequence identified in experiments performed in vitro is markedly different from that used in vivo [8].

TOPOISOMERASES: INTERACTION WITH CYTOTOXIC DRUGS

Some of the most clinically effective cytotoxic drugs kill cells by interacting with topoisomerases I and II (Table I). Drugs widely used clinically that interact with topoisomerase II can be roughly divided into intercalating agents (e.g., doxorubicin) and non-intercalating epipodophyllotoxins (e.g., VP16) [10–15]. Only one topoisomerase I inhibitor, topotecan, is currently under clinical trial, though there is evidence that the intercalator dactinomycin interacts with both topoisomerase I and II [16–19]. Topoisomerase inhibitors act by binding to the enzyme/DNA complex at the strand cleavage stage (Fig. 3). The presence of the drug halts the enzyme at this stage. The reaction cannot progress through the religation and enzyme release stages, leaving the DNA with a permanent strand break [10–15] and the presence of multiple DNA strand breaks leads to cell death. The exact mechanism by which this leads to cell death is poorly understood. There is, however, evidence that induction of apoptosis plays a role in killing by topoisomerase II inhibitors, and that interruption of the progress of replication forks is important in killing by topoisomerase I inhibitors [20–26]. It is important to appreciate that cytotoxicity is *not* due to cellular depletion of topoisomerase II, but is due to the DNA damage induced by the association of drug with enzyme and DNA.

TOPOISOMERASES: DRUG RESISTANCE AND SENSITIVITY

The level of topoisomerase in a laboratory cell line is an important determinant of the sensitivity of that cell to killing by topoisomerase inhibitors [27]. Because the topoisomerase inhibitor drug interacts with the enzyme to cause DNA damage that kills the cell, higher levels of topoisomerase lead to more DNA damage and hence better killing by the drug. Levels of topoisomerase II vary through the cell cycle, with activity increasing during S and G2 phases and peaking late in G2/M, so levels are high in rapidly proliferating cells [28–30]. This is consistent with the observation that proliferating cells are more susceptible to killing by topoisomerase II inhibitors [27]. Levels of topoisomerase II are also increased in cells that have undergone malignant transformation [31] and this may contribute to the ability of topoisomerase II inhibitors to kill malignant cells more effectively than normal cells, allowing these drugs to be used therapeutically.

The converse of this observation is that cells with low levels of topoisomerase II would be expected to be relatively resistant to killing by topoisomerase II inhibitors, and this has been demonstrated in vitro [32,33]. The most frequent mechanism for acquired resistance to

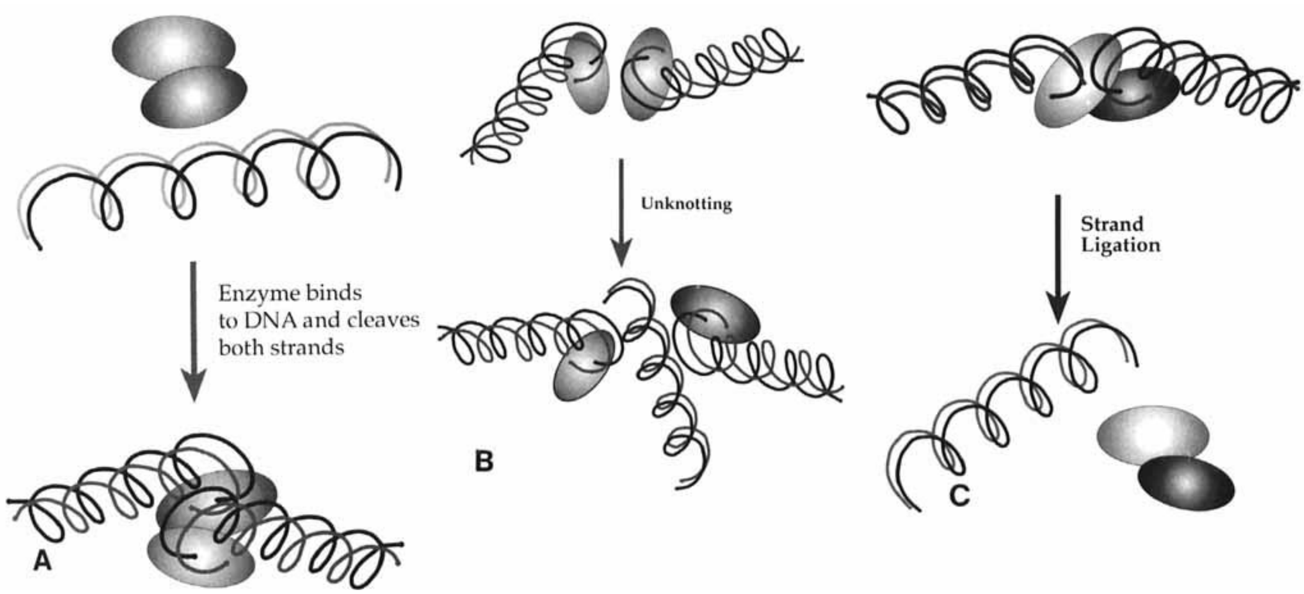

Fig. 2. The topoisomerase reaction takes place in three stages. **A:** The enzyme, which consists of two identical subunits, binds to the DNA and makes a break in the sugar-phosphate backbone. **B:** The two subunits of the enzyme separate allowing the DNA to "swivel," relieving torsional stress, or allowing a second strand of DNA to pass through in an "unknotting" reaction. **C:** The two enzyme subunits come together again and religate the break in the DNA backbone. The reaction is normally completed in seconds.

TABLE I. Drugs That Inhibit Topoisomerases I and II

Enzyme	Drug	Drug class
Topoisomerase II	Doxorubicin	Intercalating Agents
	Daunorubicin	
	4-Epidoxorubicin	
	Idarubicin	
	Mitoxantrone	
	MAMSA	
	Etoposide	Epipodophyllotoxins
	Teniposide	
Topoisomerase I	Topotecan	Camptothecin
Topoisomerase I and II	Dactinomycin	Intercalating Agent

topoisomerase II inhibitors demonstrated in the laboratory is increased expression of the multidrug resistance gene [34–39]. However, acquired alterations in topoisomerase II, including reduced levels of expression and mutations in the protein, clearly also contribute to drug resistance and failure of chemotherapy in in vitro models [40–46].

From these data it might be expected that it would be a simple matter to predict the efficacy of topoisomerase II inhibitors in vivo by measuring levels of topoisomerase in each individual tumor. While in some clinical circumstances this is broadly true, in other studies the situation has proved to be more complex. Potmesil and co-workers have demonstrated a very low level of topoisomerase II in leukemic cells from patients with chromic lymphocytic leukemia, which correlated with low in vitro and in vivo sensitivity to topoisomerase II inhibitors [47]. In contrast, Kaufmann and co-workers studied topoisomerase levels in malignant cells of patients with acute myelogenous leukemia and correlated these data with drug sensitivity [48]. While the overall level of topoisomerase II in the leukemia samples varied over a greater than 20-fold range, this did not correlate with drug sensitivity in vitro or in vivo. This may in part be due to the marked cell-to-cell variation in topoisomerase II expression seen in the leukemia cells, which may itself be related to the position of the cells in the cell cycle. Currently, further

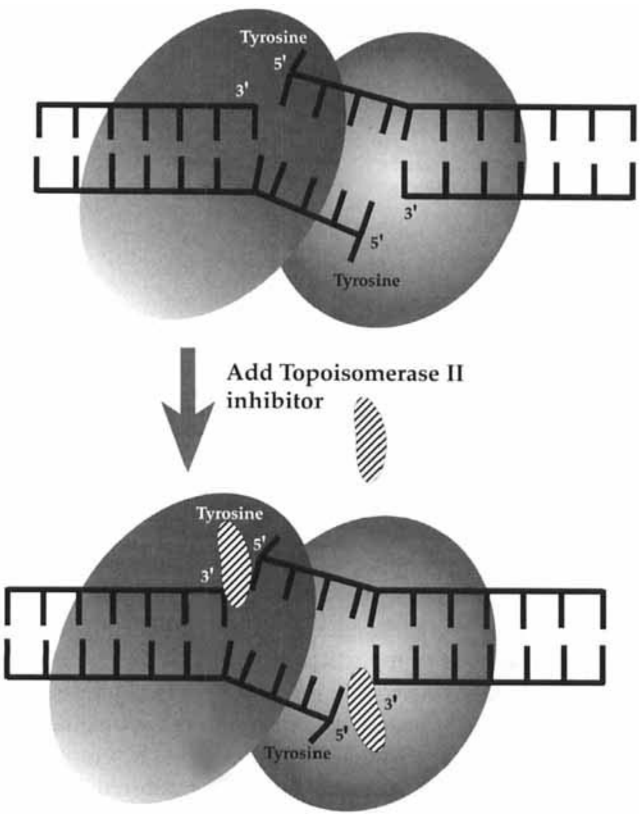

Fig. 3. Topoisomerase inhibitor drugs bind to the DNA enzyme complex at the strand cleavage stage and prevent the reaction from passing through the strand passage and religation stages.

work is needed before measurement of topoisomerase levels can be used to individualize therapy.

TOPOISOMERASES: SECONDARY LEUKEMIA

Secondary malignancy is one of the most dreaded long term complications of treatment with cancer chemotherapy. Recent studies have demonstrated two main classes of cytotoxic drugs that induce secondary hematological malignancy: alkylating agents and topoisomerase II inhibitors [49,50]. The leukemias induced by each class of drugs are commonly myeloid and have distinctive clinical and cytogenetic features.

The leukemogenic potential of alkylating agents has been known for many years and almost all alkylating agents in clinical use have been shown to increase the risk of leukemia [49,51,52]. With these drugs, the risk of leukemia appears to increase with increasing patient age, as does the risk of de novo myeloid leukemia in the population. Risk also increases with increasing cumulative dose of drug. Dose schedule dependence has not been reported. The latent period of development of leukemia following alkylating agent therapy ranges from 2–8 years. Patients commonly present with myelodysplasia and evolve to acute myeloid leukemia [49]. These leukemias characteristically have deletions of all or part of chromosomes 5 and 7 and have a poor response to therapy [52].

One of the earliest suggestions that topoisomerase II inhibitors were leukemogenic came from a study of children treated with doxorubicin, teniposide, and cyclophosphamide for non-Hodgkin's lymphoma or T-cell leukemia [53]. In this study, eight of 261 children developed a second malignancy, which was hematological in six, giving an actuarial incidence of 7.8% at 7 years. Further evidence for a role for topoisomerase II inhibitors in secondary leukemia subsequently came from studies of adults and children treated for a variety of leukemias and solid tumors. Data from the larger reported series are presented in Table II. It is notable that the two studies of germ cell tumors suggest that there may be a dose intensity effect, with the risk of leukemia remaining low in patients receiving $\leq$2,000 mg/m^2 etoposide, but increasing rapidly in patients receiving higher doses [55,56].

An important series of studies from St. Judes Children's Research Hospital has demonstrated that the risk of secondary leukemia associated with epipodophyllotoxins is related to the intensity of the dosing schedule [54]. These studies included 734 consecutive children with ALL who attained remission and received maintenance therapy according to different schedules of epipodophyllotoxin treatment. In this study the overall cumulative risk of secondary AML was 3.8% (Table II). Within the subgroups treated twice weekly or weekly with epipodophyllotoxins, the cumulative risks were 12.3% and 12.4%, respectively. The highest cumulative risk was 1.6% in the subgroups not treated with epipodophyllotoxins, or treated with them only during remission induction or every 2 weeks during maintenance treatment. After adjustment for treatment frequency, there was no apparent independent effect of the total dose of epipodophyllotoxin, in contrast with the dose effect seen with alkylating agent-induced leukemia.

As additional cases of secondary leukemia following topoisomerase II inhibitor therapy are reported, it has become apparent they are clinically and cytogenetically distinct from alkylating agent-induced leukemia. The leukemias that follow topoisomerase II inhibitor therapy typically have a short latent period (from less than 1 year to around 2.5 years), are less likely to have a myelodysplastic phase, and the overt leukemia is frequently FAB type M4 or M5 [50]. Cytogenetic studies have shown that these leukemias commonly have structural abnormalities at the 11q23 locus, usually in the context of a balanced translocation such as t(9:11), t(19:11) or t(4:11) [50].

Considerable attention has been given to the frequent

TABLE II. Clinical Studies Demonstrating Involvement of Topoisomerase Inhibitors in Secondary Leukemia

Primary cancer	Topo II inhibitor therapy[a]	No. treated	No. with 2° leukemia	Cumulative risk (%)	Reference
ALL	VM26 and/or VP16	734	21	3.8 @ 6 yrs	54
NHL/T-cell leukemia	Doxorubicin/VM26	261	6 (+ astrocytoma, n = 1; sarcoma, n = 1)	7.8 @ 7 yrs	53
Germ cell tumors	VP16 (2,000 gm/m^2)	212	5	4.7 @ 5.7 yrs	55
Germ cell tumors	VP16 (2,000 gm/m^2)	538	2	0.37 @ 4.9 yrs	56
ALL	VP16	205	10	5.9 @ 4 yrs	57
Childhood solid tumors	VP16/VM26 (in subset of patients only)	3,365 (all therapies)	12	1.3 @ 10 yrs	58
ALL	None used	752	2	N/A	59
Lung cancer	VP16/doxorubicin	796	6	14 @ 4 yrs	60
Breast cancer	Mitoxantrone	71	5	25 @ 37 mos	61
Breast cancer	4 Epidoxorubicin	74	3	16 @ 33 mos	62

[a]VP16 = etoposide; VM26 = teniposide.

occurrence of structural abnormalities of the chromosomal region 11q23 in topoisomerase II inhibitor associated secondary AML. Studies of both de novo and therapy-related leukemias by a number of laboratories have identified the gene located at 11q23 that is disrupted in translocations with a variety of different partner chromosomes. The gene has been designated MLL-1, ALL-1, HRX, and HTRX by different groups and is thought to be the human counterpart of the drosophila gene trithorax, a regulator of transcription [63–66]. In acute leukemias with 11q23 aberrations, the chromosomal translocations result in an in-frame (i.e., able to be expressed) gene fusion between the amino-terminus of the MLL-1 gene and a partner gene on the translocated chromosome. Fused transcripts derived from both genes can be detected, and are presumed to lead to leukemogenesis. Translocations between MLL-1 and at least 15 different chromosomal bands have been described, suggesting that the MLL-1 derived sequences on 11q23 are primarily responsible for leukemogenesis [66–70].

Although a lot of interest has been aroused by the finding of 11q23 abnormalities, other balanced translocations seen commonly in de novo AML are also seen frequently in secondary AML related to topoisomerase II inhibitor therapy, including t(8:21) and t(15:17) [71–74]. It is notable that the abnormalities of 11q23 seen in topoisomerase II inhibitor related secondary AML are commonly balanced translocations, while those seen after exposure to alkylating agents are more commonly unbalanced [71]. Topoisomerase II is an attractive candidate as a mediator of illegitimate recombination events that generate chromosomal translocations. During the strand passage stage of the topoisomerase II reaction there are two available "sticky" ends of DNA, which are generally rapidly and accurately religated. However, if an error in religation occurs, the DNA ends can be religated to incorrect partners (an illegitimate recombination event) and a translocation can be generated. There is experimental evidence that topoisomerase II can mediate illegitimate recombination events, thus supporting this hypothesis [75]. It seems therefore plausible that interference with the progress of the topoisomerase II reaction inhibitor drugs could lead to errors in religation and the production of a translocation. Intensive scheduling of topoisomerase II inhibitors may allow insufficient time for repair or elimination of cells with significant but sub-lethal chromosomal damage, and so be more likely to induce leukemia.

To prove that topoisomerase II is playing a role in the genesis of secondary AML and de novo AML, workers have sequenced the breakpoint region in the MLL-1 gene to try to identify topoisomerase II preferred cleavage sites. Study of 10 de novo acute leukemias revealed topoisomerase II consensus sites at the breakpoint in only 3 of 10, even after allowing up to 3 mismatches [76]. However, examination of the breakpoint region on chromosome 11 in a single case of infant AML, in which cytogenetics revealed t(9:11), showed topoisomerase II consensus cleavage sites adjacent to the breakpoint [77]. Interpretation of these data as supporting or excluding a role for topoisomerase II in the etiology of translocations should be viewed with caution as the vertebrate consensus sequence was derived in vitro, and its applicability in vivo cannot be assumed. Topoisomerase binding at sites other than those described as consensus sites can likely occur, though maybe at a low frequency. Such cleavage may be sufficient to generate balanced translocations at non-consensus sequence sites in some circumstances. Conversely, it must be remembered that the topoisomerase II consensus sequence is highly degenerate. It occurs frequently throughout the genome, perhaps as frequently as every 700 base pairs, so the presence of such a site may simply be fortuitous.

In addition to possible topoisomerase II activity in the region of translocation breakpoints, there is also some evidence of recombinase activity. Recombinases are

cellular enzymes that normally mediate the DNA recombination events required for immunoglobulin gene rearrangements. DNA sequencing has revealed signs suggesting recombinase activity, such as heptamer and nonamer recombinase recognition sites and evidence of terminal deoxynucleotide transferase activity. In a small number of cases of primary and secondary leukemia, such evidence of recombinase activity was found at the translocation breakpoint. This suggests that this enzyme, either alone or together with topoisomerase II, may contribute to the production of the translocation [77,78].

It is clear that the generation of specific balanced translocations is a pivotal event in leukemogenesis in a subset of both primary and secondary leukemias. It is likely that translocations are caused by a variety of different mechanisms, but the evidence indicating that interference with the topoisomerase reaction is important in the generation of secondary AML is strong. The translocations seen in primary AML are the same as those in secondary AML associated with topoisomerase II inhibitors, so that similar etiologic mechanisms may be operative in at least some of these primary leukemia cases [79]. In de novo AML, topoisomerases might generate balanced translocations by a spontaneous error in the reaction or, more speculatively, by interaction with dietary topoisomerase II inhibitors such as caffeine, 8-methoxycaffeine, or genistein, a flavone that is present in soy [80–83].

In summary, topoisomerases are essential enzymes that perform complex and vital tasks in DNA metabolism. These enzymes provide a target for therapeutic cytotoxic drugs that halt the topoisomerase reaction at the strand cleavage stage; and, through this interaction, cause DNA damage and cell death. Dose-intensive schedules of treatment with topoisomerase II inhibitor drugs are associated with high rates of drug-induced leukemia, frequently associated with balanced chromosomal translocations. Interference with the topoisomerase reaction, leading to the generation of chromosomal translocations may be a common mechanism of leukemogenesis in both de novo and therapy-related AML.

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